

Immunofluorescence

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 An abbreviated version of this protocol was published in eLIFE in Apr 2016

Blockade of glucagon signaling prevents or reverses diabetes onset only if residual β -cells persist

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Detailed protocol

- Starting material: cryosections, 10 μ m-thick.
- Rehydration: 15 min at RT
- Permeabilization: 20 min at RT in PBS-Triton 0.1%
- Wash 3 x 5 min in PBS
- Blocking: 30 min at RT in blocking buffer (3% BSA, 0.1% Tween in PBS)
- Primary antibodies: 2h at RT

Host	Target	Dilution	Vendor
Guinea pig	Insulin	1:400	DAKO
Mouse	Glucagon	1:250 to 1:1000	Sigma
Rabbit	GFP	1:200	Molecular Probes Inc.

- Wash 3 x 5 min in PBS
- Secondary antibodies: 45 mn at RT

Host	Target	Dilution	Fluorophore	Vendor
Goat	Guinea Pig	1:500	AF568	Molecular Probes Inc.
Goat	Mouse	1:500	AF647	Molecular Probes Inc.
Goat	Rabbit	1:500	AF488	Molecular Probes Inc.
-	DAPI	1:500	-	ThermoFisher

- Wash 3 x 5 min in PBS
- Mount with mounting media and coverslip

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Damond, N. (2020). Immunofluorescence. Bio-protocol Preprint. bio-protocol.org/prep249.
2. Damond, N., Thorel, F., Moyers, J. S., Charron, M. J., Vuguin, P. M., Powers, A. C. and Herrera, P. L. (2016). Blockade of glucagon signaling prevents or reverses diabetes onset only if residual β -cells persist. eLIFE. DOI: [10.7554/eLife.13828](https://doi.org/10.7554/eLife.13828)

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